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Insights into the role of interferon lambda in hepatitis C virus infection

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Infection with hepatitis C virus (HCV) is characterized by two different outcomes. Approximately 30% of HCV-infected patients spontaneously resolve HCV infection while the others fail to control the infection. Once chronic HCV infection is established, antiviral therapy based on pegylated interferon alpha (PEG-IFNα) and ribavirin achieves viral clearance in only 30–50% of cases [6]. Genome-wide association studies (GWAS) indicated that host genetic variation plays a key determinant in natural and treatment-induced control of HCV infection. Independent GWAS studies consistently identified variants within the *IL28B* gene region that are strongly associated with spontaneous HCV clearance and hepatitis C treatment-induced viral clearance [4,10,13,18–20]. However, the mechanism by which *IL28B* variants influence viral clearance remains undetermined. The *IL28B* gene forms a cytokine gene cluster with *IL28A* and *IL29* on human chromosome 19. *IL29*, *IL28A*, and *IL28B* are also known as *IFNλ1*, *IFNλ2*, and *IFNλ3*, respectively. They are type III IFNs with antiviral activity. *IL28B* is highly homologous to *IL28A* (96%) and shows 81% homology with *IL29*. Type III IFNs have biological activities that are similar to those of type I IFNs (IFNα/β), although they share very little sequence homology. Type III IFN expression depends on the same stimuli (viral infection, Toll-like receptor ligands) and signal transduction pathways as those involved in type I IFN expression, which leads to the induction of several hundred interferon-stimulated genes (ISGs), such as *Mx1*, *OAS* or *IFIT* [7,16] (Fig. 1).

In the current issue of the Journal of Hepatology, Langhans et al. performed a cross-sectional analysis of IFNλ serum levels in HCV-infected patients with different outcomes and stratified their results with the single nucleotide polymorphism (SNP) rs12979860 that is located 3 kb upstream of the *IL28B* gene. GWAS studies carried out by Ge et al. [4] and McCarthy et al. [10] found a striking correlation between carriers with the rs12979860 CC genotype and sustained virological response (SVR) rates. The protective rs12979860 CC genotype was not only associated with significantly higher SVR rates in patients with chronic HCV infection but also predicted spontaneous HCV clearance [20]. The study by Langhans et al. included 60 treatment-naive patients with chronic HCV infection, 19 patients with acute HCV infection, 29 patients with spontaneous HCV clearance, and 26 healthy individuals. The authors found that IL28 A/B and IL29 serum levels were significantly higher in carriers of the protective rs12979860 CC genotype than in carriers of the TT genotype when all study groups were analyzed as a single population. Because IL28A/B serum levels were rather low or undetectable, the authors focused exclusively on IL29 in their subsequent studies. When IL29 serum levels were analyzed with respect to the different outcomes, patients with chronic HCV infection displayed significantly lower IL29 serum levels than patients with acute hepatitis C, patients with self-limited HCV, and healthy individuals. IL29 serum levels were consistently and significantly higher in patients with resolved HCV infection than in patients with chronic HCV infection, even when the IL29 serum levels were stratified for the different rs12979860 genotypes. Next, Langhans et al. conducted a prospective study of serum samples from patients who had acute hepatitis C. The authors found that patients who resolved their acute infection had significantly higher IL29 serum levels than patients who progressed to chronicity. When IL29 serum levels were stratified with the different rs12979860 genotypes, carriers of the CC genotypes had consistently higher IL29 levels than carriers of the TT genotype. Finally, there was a trend ($p = 0.053$) to higher IL29 levels in HCV resolver with the protective rs12979860 CC genotype than to HCV non-resolver with the CC genotype. Accordingly, the authors concluded that high IFNλ levels may predispose to HCV clearance and that the rs12979860 CC genotype may contribute to viral clearance due to enhanced IFNλ expression.

The work by Langhans et al. provides initial insights into the role of IFNλ in HCV clearance and the functional consequences of the *IL28B* polymorphism. The strong association between high IL29 serum levels and spontaneous HCV clearance suggests that IL29 has unique antiviral effects that are important for the control of HCV infection. In fact, IFNλ exerts antiviral effects on HCV replication with kinetics and efficiency similar to IFNα, as shown in the HCV replicon system and the infectious HCV cell culture model [9,14]. However, IFNλ treatment could not entirely eliminate HCV replication, suggesting that IFNλ induced genes fail to completely abrogate viral replication. IFNλ signaling is initiated through a membrane receptor system distinct from that of IFNα. The IFNλ receptor complex consists of IFNLR1 (IFNλ receptor 1; also known as IL-28Rα) and IL-10 receptor 2 (IL-10R2), while

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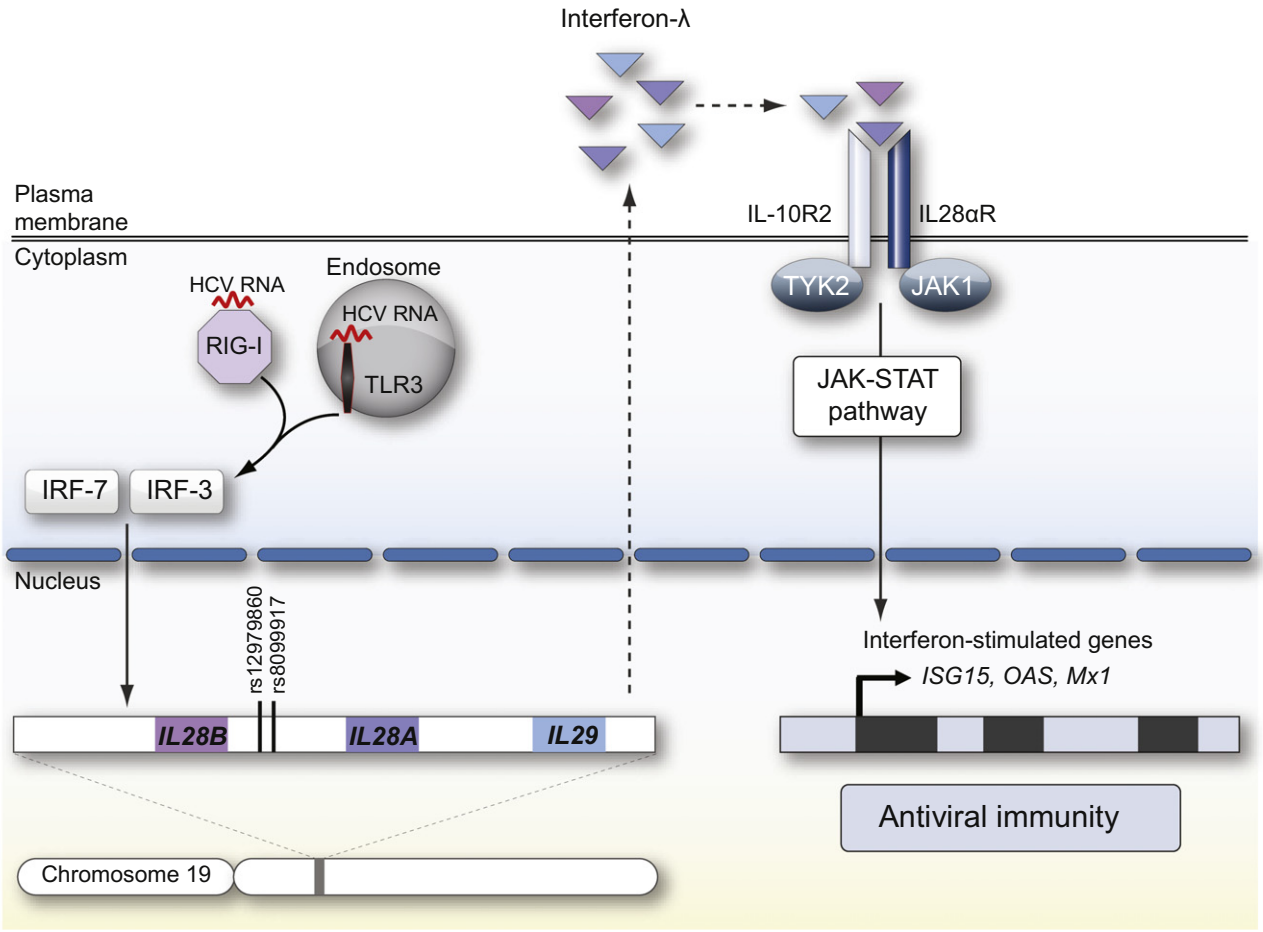

Fig. 1. Interferon lambda pathway. HCV-infected cells recognize viral RNA through cellular sensors, e.g. Toll-like receptor 3 (TLR3) and cytoplasmic receptor RIG-I. Stimulation of these receptors leads to the activation of transcription factors such as interferon-regulatory factor 3 (IRF-3) and IRF-7. Binding sites for IRF-3 and IRF-7 have been identified in the promoter region for *IFNλ* and are essential for IFNλ transcription. The three IFNλ subtypes (*IL28A*, *IL28B*, and *IL29*) exert their activity through a receptor composed of interleukin-10 receptor (IL-10R2) and IFNλ receptor 1 (IFNLR1 or IL28αR). This results in the activation of the JAK-STAT pathway and the induction of interferon-stimulated genes (ISGs), e.g. *ISG15*, *Mx1*, and *OAS*. ISGs products have been shown to interrupt HCV replication through processes that include suppression of viral replication and protein synthesis. Polymorphisms upstream of the *IL28B* gene (rs12979860, rs8099917) are associated with spontaneous and treatment-induced HCV clearance. *IL28B* polymorphism appears to affect the expression of IFNλ and ISGs by pathways that are yet unknown.

the IFNα receptor is constructed from the ubiquitously expressed IFNAR1 and IFNAR2c subunits [7]. Unlike the response to IFNα, the response to IFNλ appears to be restricted to epithelial cells and is notably absent in most hematopoietic cells due to weak or absent IL28Rα expression [21]. Surprisingly, in mice, the liver responded poorly to IFNλ and expressed low amounts of IL28Rα [14,17], suggesting that the IFNλ response plays only a minor role in the host defense against viruses that infect the liver. However, Doyle et al. detected IL28Rα protein expression in the livers of HCV-infected patients, as well as transcriptional induction of *OAS* and *Mx1* following IL29 stimulation of primary human hepatocytes [2]. Furthermore, a recently completed phase 1b study with PEG-IL29 in patients who were chronically infected with HCV genotype 1 showed promising results. IL29 was well tolerated without the toxicities typically associated with IFNα and exhibited potent antiviral activity [12]. IFNα treatment triggers flu-like symptoms, depression, and hematopoietic side effects. The greater cell-specific expression pattern for the IFNλ receptors may explain the fewer side effects observed during IFNλ treatment. Although these studies support the view that IFNλ possesses antiviral effects against HCV, further research is needed to determine the precise role of endogenous IFNλ in HCV clearance and to evaluate how much antiviral defense is added by the IFNλ system to the very potent IFNα/β system.

Cytokines are powerful mediator and communication molecules. However, the significance of cytokine levels in the serum is often difficult to interpret. With few exceptions, the biological half-life of cytokines *in vivo* is short. The short *in vivo* half-life is the result of binding to high-affinity receptors or plasma proteins, as well as of proteolytic degradation. It was, therefore, surprising that Langhans et al. detected IL29 at steady state levels and that high levels of this cytokine turned out to be an early predictor of HCV clearance. The data are difficult to reconcile with the observation that patients who respond poorly to IFNα therapy have a high pre-therapy level of intrahepatic ISG expression [1,3,15]. It will be, therefore, important to determine the spectrum of ISG expression in HCV-infected patients with elevated IL29 levels. IFNs are induced to varying degrees in most cell types, with mye-

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loid and plasmacytoid dendritic cells (mDCs and pDCs) being the two most prominent sources of these cytokines. IFNs released by DCs not only have potent antiviral activity but also support subsequent steps of antiviral immunity, such as the activation of natural killer and T cell-mediated cytotoxicity. Previous studies reported that HCV interferes with the IFNα production in pDCs (for review, see [8]). Additional experiments by Langhans et al. suggest that HCV also affects IFNλ production in mDCs and pDCs. The presence of the HCV envelope glycoprotein E2 and NS3 protein, but no other recombinant HCV proteins, significantly reduced IL29 production by DC subsets following stimulation with TLR3 and poly I:C. The finding that HCV might have developed strategies to attenuate the IL29 response in DCs underscores the importance of this cytokine for antiviral protection. Studies with the infectious HCV cell culture model will be required to demonstrate conclusively that HCV counteracts the IFNλ response in DCs and to investigate the underlying mechanism. IFNλ may also act on DCs through an autocrine feedback loop. Diverse immunomodulatory effects of IFNλ on DCs have been reported, including maturation and differentiation of DCs [11]. Therefore, it will be very interesting to investigate whether HCV-induced impairment of IFNλ production has an impact on the HCV antigen presentation function by DCs and the generation of antiviral cellular immune responses.

Polymorphism may have a functional role by influencing promoter activity (gene expression), messenger RNA (mRNA) conformation (stability), and protein stability (half life). Since the identified SNPs are quite far from the *IL28B* gene, the direct functional role of *IL28B* polymorphisms on *IFNλ* gene expression and function is uncertain. At the present, *IFNλ* gene expression data have yielded conflicting results (Table 1). Langhans et al. found significantly higher IL28A/IL28B and IL29 serum levels in carriers of the protective rs12979860 CC genotype. Ge et al. [4] found no association between rs12979860 CC genotype and increased *IL28B* mRNA expression in peripheral blood mononuclear cells (PBMCs). Suppiah et al. [18] and Tanaka et al. [19] reported that SNP rs8099917, a SNP 8 kb upstream of the *IL28B* gene and associated significantly with treatment failure, was associated with lower *IL28B* mRNA expression in PBMCs. Honda et al. [5] examined *IL28B* mRNA expression and ISG induction in the livers of patients who had chronic HCV infection. They did not find significant differences in hepatic *IL28B* mRNA expression between carriers with rs8099917 risk and protective genotypes. However, hepatic ISG induction was significantly higher in patients who had the rs8099917 risk genotype than in those who had the protective genotype. This suggests that the *IL28B* polymorphism differentially regulates the expression of ISGs in the liver. Unfortunately, the currently available data are insufficient to firmly delineate the effect of the *IL28B* polymorphism on IFNλ expression. Further studies are needed to clarify the impact of the *IL28B* polymorphism on the regulation of antiviral signaling pathways in hepatocytes, as well as innate immune cells, such as DCs and macrophages.

Elucidating the functional consequences of the *IL28B* polymorphism continues to be a difficult challenge. SNPs within the *IL28B* gene region appear to affect IFNλ and ISG induction by pathways that are yet unknown. A better understanding of the cross-talk between the IFNα/β and IFNλ response and the impact of IFNλ on the adaptive immune response may allow us to peek behind the curtain.

Table 1
Associations between carriage of *IL28B* risk alleles and IFNl or ISG expression.
(See below-mentioned references for further information).

	Effects of *IL28B* risk alleles on	
Compartment	IFN λ expression	ISG expression
Serum	reduced [Langhans et al. [22]]	n.d.
PBMCs (mRNA)	reduced [Suppiah et al. [18], Tanaka et al. [19]]	n.d.
	unchanged [Ge et al. [4]]	n.d.
Liver (mRNA)	unchanged [Honda et al. [5]]	increased [Honda et al.[5]]

n.d. = not determined.

Conflict of interest

The author declared that she does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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